A STUDY IN MICROTOMY FOR ELECTRON MICROSCOPY

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SIX FIGURES

INTRODUCTION

The importance of ultrathin sectioning to electron microscopy of biological material, particularly whole tissues, was recognized more than a decade ago by von Ardenne ('39), who at the time devised techniques for cutting thin wedge-shaped sections. Thereafter progress in the development of better procedures was slow until relatively recent years when a number of discoveries bearing on embedding matrices for the tissue, cutting edges and microtomes have combined to make the procedures practical and effective. Thus, for embedding the object to be sectioned, the methacrylate plastics (Newman, Borysko and Swerdlow, '49) seem to have replaced other material such as paraffins or paraffin-celloidin combinations. The glass knife (Latta and Hartmann, '50) has found some favor over the specially sharpened steel edge and already a number of microtomes or modifications of previously existing microtomes have been found capable of cutting adequately thin sections (see Fullam and Gessler, '46; Pease and Baker, '48; Newman, Borysko and Swerdlow, '49; Hillier and Gettner, '50; Geren and McCulloch, '51; Cocks and Schwartz, '52; Watson, '53; Sjöstrand, '53). It is the purpose of this report to describe two additional microtomes and to point out certain of their virtues and accomplishments. We would not consider their description important if they had not during

one and one-half years of laboratory use succeeded in two important respects: (a) in consistently providing ribbons and therefore serial sections approximately 50 mμ thick (or more or less if desired), and (b) in performing satisfactorily in this and other laboratories for a number of persons who had not had much previous experience.

With these microtomes and the supplementary procedures currently available, ultrathin sectioning seems to us as straightforward and productive for its own purposes as sectioning from paraffin or celloidin is for optical microscopy.

The instruments to be described have two features which more than any others seem responsible for their successful operation. First, the block from which sections are being cut passes the knife only on the cutting stroke. This eliminates the tendency otherwise encountered for the face of the block, on the return stroke, to pick up the preceding section, a tendency especially pronounced with microtomes in which the block is continuously advanced toward the knife. It prevents, moreover, contamination and possible compression of the block face. The values of this "single-pass" design were demonstrated by an earlier model of microtome built by one of us (J. B.) (see Claude, '48) in which the specimen was fastened to the edge of a disc or wheel and revolved past the knife. This construction has since been duplicated in instruments described by Gettner and Ornstein ('53), Watson ('53) and Sjöstrand ('53). While the revolving disc is not a component of these new microtomes described below, the same "single-pass" motion of the block is achieved by other means.

A second significant feature of the microtome is the essential absence of oil-lubricated surfaces in the moving parts. Thus in operation there are no oil films to stabilize or conversely to vary in thickness. Certain other structural aspects of the instrument which doubtless contribute to its useful functioning are mentioned in what follows.

The microtomes to be described (especially the one referred to as the "improved" model) resemble in some features the

Cambridge Rocking Microtome which has long been used in the preparation of sections for optical microscopy and more recently for electron microscopy (Bretschneider, '49). The design of the latter instrument does not, however, provide

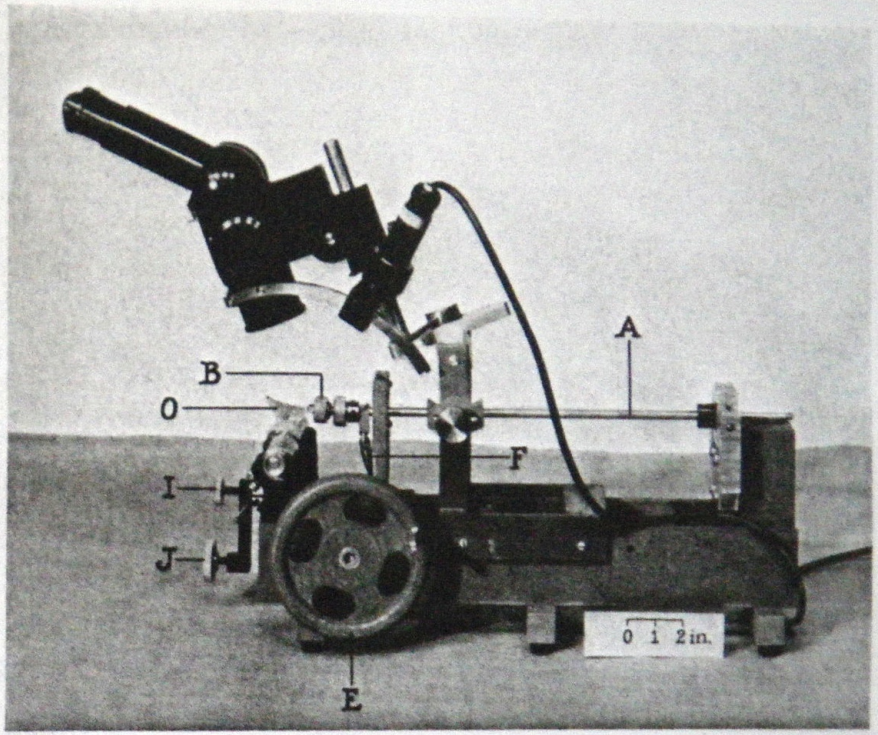

Fig. 1 Side view of microtome constructed as experimental model. The horizontal bar (A) which has a specimen holder at one end (B) is drawn vertically past the knife and bath (O) by a connecting shaft (F) attached to an eccentric. The motion is actuated by the hand-driven wheel (E). Thumb screws (I) and (J) are respectively coarse and fine adjustments designed to move the knife toward the specimen block for trimming and cutting thick sections. When cutting ultrathin sections the specimen is advanced toward the knife by heating the bar (A). The dissecting microscope and lamp are useful for observing the quality of the sections as they leave the cutting edge and float out on 10% acetone in receiving bath (O).

for cutting sections thinner than 200 mμ. In some respects also the microtomes reported on here are similar to an instrument, devoid of movable bearings, which was observed in experimental stages of construction in H. S. Bennett's laboratory (University of Washington) in 1951. In it, the

specimen was supported on the end of a bar and brought past the cutting edge by simply flexing the bar. It had no provision for avoiding the knife on the return stroke and for this and other reasons did not prove satisfactory in its original form.

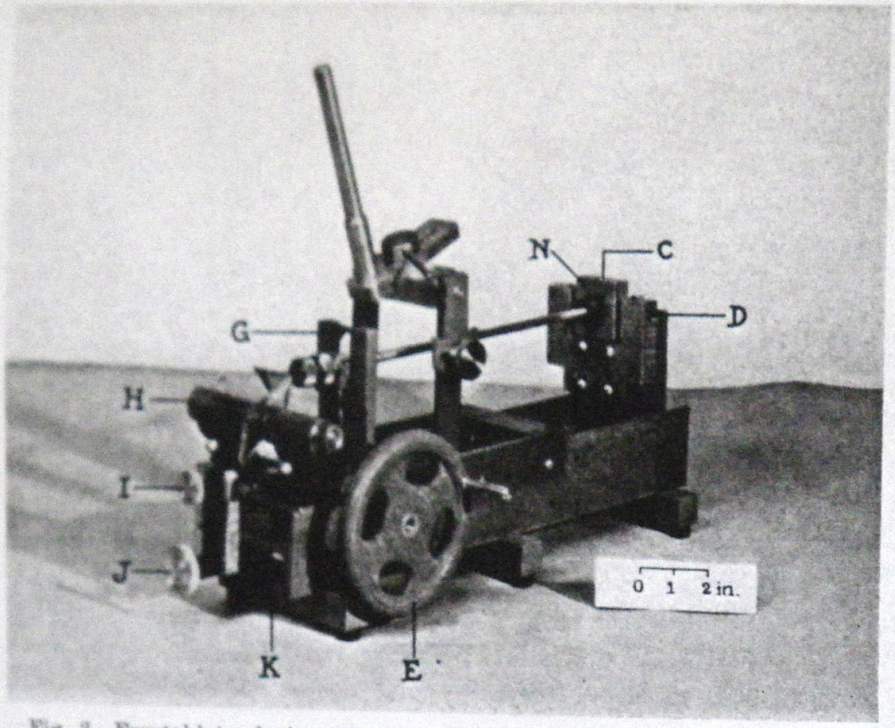

Fig. 2 Frontal-lateral view of microtome to show especially the pivot mount for the back end of the horizontal bar and the guide (G). The rectangular pivot block (N) is supported by a pair of pivots, one in from (D) and the other directly opposite. Likewise a pair of pivots arranged vertically under (C) supports the back end of the horizontal bar. The former pair of pivots provides for the vertical motion of the bar and so the cutting and return stroke. The latter or vertical pair of pivots (C) provides for the horizontal motion of the bar and permits it to be swept to one side of the knife on the return stroke. Friction at the pivots holds the bar in any position it is placed. This accounts for the effectiveness of the guide (G), which takes the bar to the left at the bottom of the down or cutting stroke where it remains on the up stroke until guided back to a position over the knife. The glass knife is supported in a stout brass bar (H) by a clamp that may be tightened by the screw on the right-hand end of the bar. The knife holder is held rigidly to the base by a clamp actuated by lever (K).

Description of the prototype. The experimental model and prototype for the later model is pictured in figure 1. The working parts are built upon and fastened to a 4-inch I beam which gives to the whole mechanism an essential degree of rigidity. Above this base and horizontal to it is supported a 5/16-inch duraluminum bar (fig. 1, A). One end of this bar (fig. 1, B) is designed to hold the specimen while the other end is supported in a double set of pivots (fig. 2, in from C and D). These latter provide for the vertical and lateral motions which the rod must make when it is activated by turning the wheel at E (figs. 1 and 2) and they are kept just tight enough with set screws (fig. 2, C and D) to create sufficient friction to hold the bar wherever it is guided. An eccentric (not visible) and connecting rod (fig. 1, F) move the bar vertically and the frame immediately back of the specimen holder (fig. 2, G) guides it laterally. The specimen holder (fig. 1, B) is built like a chuck and is designed to take plastic blocks polymerized in 00 gelatin capsules (8 mm o.d.). This chuck is in turn supported in a ball-and-socket joint which allows the axis of the holder and the plastic-embedded specimen to be oriented at any angle up to 30° with respect to the longitudinal axis of the horizontal rod.

The knife, in this case glass with an angle of 45° at the cutting edge, is supported in a stout brass rod (fig. 2, H) which is in turn held at each end in sturdy uprights of conventional design (Spencer microtome). The construction here is similar to that described by Latta and Hartmann ('50). The uprights are continuous with a base which is built to slide along a short track running lengthwise on the microtome base. Through this motion, controlled by a thumb screw (figs. 1 and 2, I), the knife edge may be advanced to the face of the plastic block. A second screw at (J) works a lever with a ratio of about 5 to 1 and provides for a more gradual forward motion of the knife for final trimming of the block and cutting thicker sections. A clamping device controlled by the bar at (K) (fig. 2) holds the knife block tight to the base when sections are being cut.

In this model of the microtome the specimen block is advanced toward the knife by thermal expansion of the horizontal pivot-supported rod (fig. 1, A). The source of heat can be varied but in our experience a 60-watt bulb in a gooseneck reading lamp at about 4 inches from the bar is all that is necessary. Any electric coils or heating ribbons attached to the bar are likely to interfere with its free motion and thus with the operation of the microtome. An infra-red lamp,

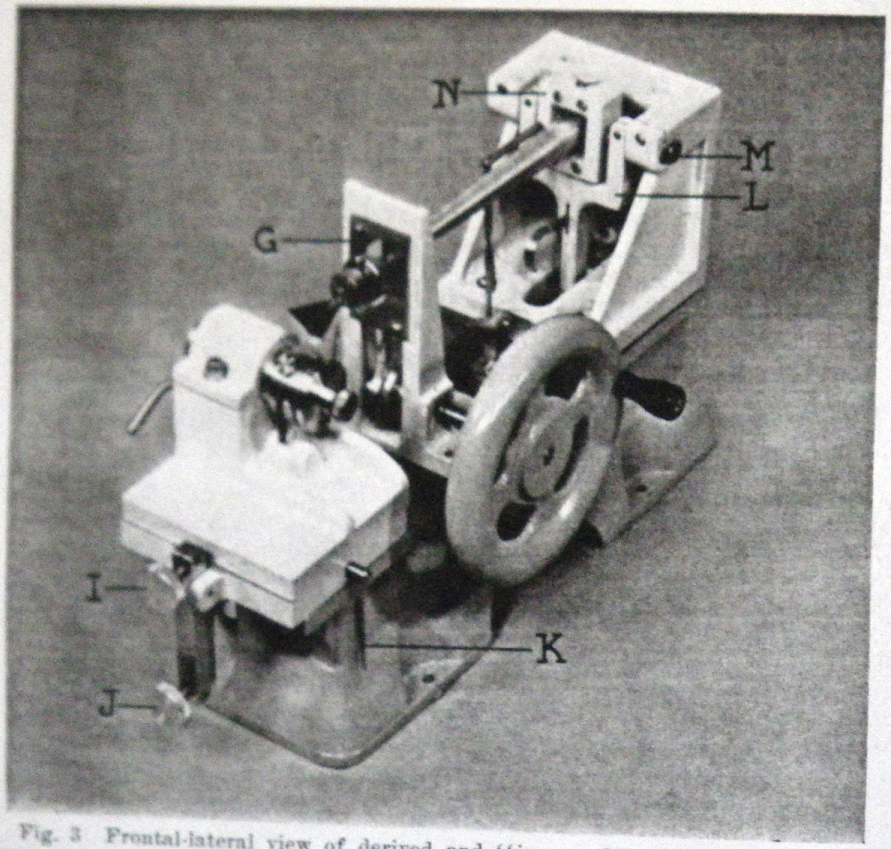

Fig. 3 Frontal-lateral view of derived and "improved" model of microtome. The general features of design are similar to those of the prototype. The single exception is the addition of a device for mechanical rather than thermal advancement of the specimen toward the knife. Features of this shown in this photograph include the rectangular Y (L) which is supported by pivots at (M) and on the opposite side. This Y carries the pivot mount (N) of the horizontal bar and provides for the forward motion of the bar as shown in detail in figures 4A and 4B. Other structural details are obvious and are labeled as in figures 1 and 2.

if adequately shielded from the base, may be used but has no great advantage over the reading lamp as a source of heat. The essential thing of course is that the bar be heated and not the base or at least that they be warmed differentially.

The mounting for a dissecting microscope as illustrated in figure 1 is not an essential feature of the instrument although some form of magnifier which can enlarge the cutting edge and sections 6 or 8 times is valuable. Such a magnifier can be just as well supported from an independent base.

The derived and "improved" model.[1] A microtome of similar design is shown in figure 3. It was constructed as an "improved" model of the prototype although in terms of section quality it has not produced anything distinctly better than the original. However, certain features of its construction make its operation more automatic and less dependent on the personal factor of the operator. It has, for example, a more sturdy mount for the knife and also for the pivots at the back end of the instrument. The horizontal bar is tapered and presumably less responsive to intrinsic and extrinsic vibrations. The major difference, however, is the inclusion of a component which mechanically advances the specimen toward the knife. It consists in the main of a Y-shaped block (figs. 3 and 4, L) suspended in pivots (M). The Y supports in its arms the pivot block (N) which provides for the universal motion of the bar. The outside pair of pivots (M) are arranged slightly above those inside (P) which support the rectangular pivot block (N) (fig. 4). The outside pivots constitute then a fulcrum and any motion of the bottom of the Y block forward or backward along the longitudinal axis of the microtome advances or retracts the bar (A). The ratio between the length of the Y (from M center to R, fig. 4) and the distance between pivot centers (M and P) is made large (200:1 in this model) so that a small forward motion of the bottom of the Y advances the specimen toward the knife only 1/200 as much. This motion of the bottom end of the Y is obtained by engaging it (at R in fig. 4B) with a finely threaded screw (S) which is turned slightly with each up-

ward motion of the horizontal bar. The mechanism accomplishing this consists of an arm (figs. 4A and 4B, T) which arises from the pivot block (N) and, with its vertical extension, duplicates the motion of the horizontal bar. This

Figs. 4A and 4B Scale drawings of front and side views of advancing mechanism which is part of microtome pictured in figure 3. It is designed to advance the horizontal bar (fig. 4B, A) at nearly even increments of 0.025 μ or multiples of the same. All the moving parts are supported in pivots which, by means of set screws, are kept tight enough to eliminate all play. The single bearing in the advancing mechanism that is not pivotal (fig. 4B, R) is under the constant tension of a coil spring (Sp_c).

One pair of these pivots (M) is fixed in the back casting of the microtome and supports the rectangular Y (L). It in turn holds in its upper arms a rectangular block (N) by horizontal pivots (P) which permit the block to tip from the vertical. The positioning of this latter pair of pivots (P) slightly below the fixed pair (M) permits the block and the horizontal bar it supports to be swung slightly forward or backward from the position of pivots (M). It is by way of this motion that the specimen is advanced toward the cutting edge. Within the frame of the rectangular block (N) the back end of the horizontal bar (A) is supported by a pair of vertical pivots which obviously allow the bar to swing in a horizontal plane. These three sets of pivots provide then for the end-to-end, horizontal and vertical motions of the bar and, accordingly, the specimen.

The rest of the device is designed to move the specimen at even increments toward the knife. It can be seen that the shaft of the rectangular Y extends down to and engages a screw (S) which is supported in pivots parallel to the midline of the instrument. The engaging tooth (R) is kept in contact with and in uniform relation to the thread of the screw by the coil spring (Sp_s) and the fiber block (E). As the screw is rotated, the arm of the Y moves forward and the rectangular block above (with the bar and specimen) moves in the same direction approximately 1/200 as much. Since the pivots (P) describe an arc in their motion with respect to the center of pivots (M), it is obvious that the forward motion of the block is a projection of the arc to the horizontal, and that sections cut with the engaging tooth at either end of the screw will be slightly thinner than those cut (at the same setting) with the engaging tooth at the center. The difference in thickness is not likely to exceed, however, that introduced by temperature variations and other instrumental factors.

The rotation of the screw is controlled by a ratchet device (W) built co-axial with the screw and is part rigidly affixed to it. The ratchet wheel is turned by a pawl (U_1), which in turn is actuated from a bar (T) which is parallel to and duplicates the motion of the cutting bar (A). The amount the ratchet is rotated may be varied from one to several teeth by the presence and control of a limiting disc (LD) and pawl (U_2) which rides on it. With the control bar (CB) far to the right, the pawl (U_2) is restrained from engaging the ratchet by pawl (U_3) until it, (U_3), is almost at the end of its range of motion and so moves the ratchet only the circumferential distance occupied by one tooth. With the control bar (CB) far to the left or at any intermediate point the movement of the ratchet is proportionately greater.

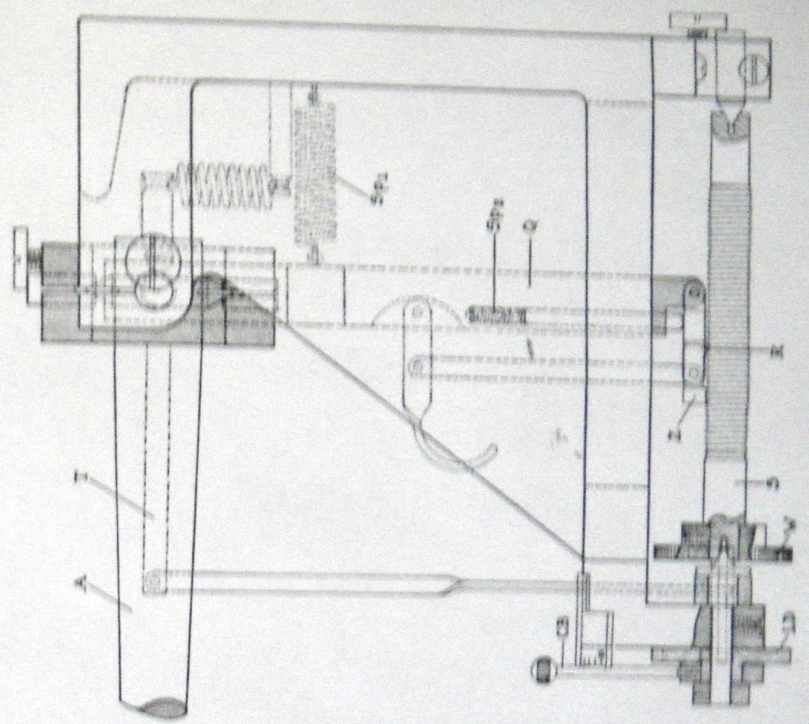

Figure 4B

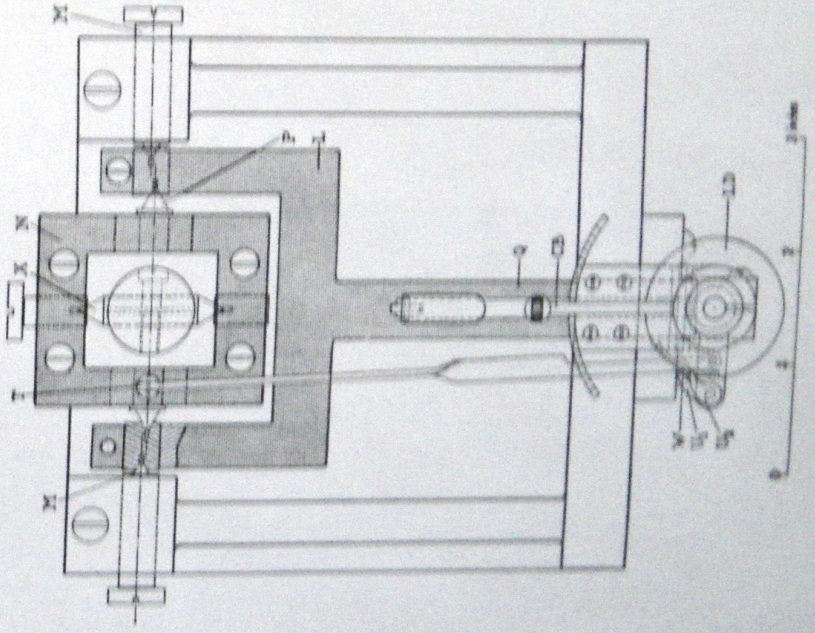

Figure 4A

connects with and acutates a pawl (fig. 4A, U_1) which in turn engages a toothed ratchet wheel (figs. 4A and 4B, W) and turns it and the screw an amount which can be varied from one to several ratchet teeth (see fig. 4 legend). Thus it is that the specimen in this model may be moved toward the knife as little as 0.025 μ. An even smaller forward motion could of course be obtained by increasing the above ratio, by reducing the pitch of the screw or by turning the screw less with each section cut. Sections thinner than 0.025 μ are seldom needed, however, and are difficult to cut from plastic blocks in any case.

The knives we have used have been obtained commercially from the Acme Glass Co., Cambridge, Mass. They have been cut from window glass, 3/16 inch thick, in the shape of a rhomboidal equilateral parallelogram with the acute angle (cutting edge) at 45°. They are approximately two inches on a side.

Microtomy. In the acquisition of suitably thin sections the quality of the microtome and the cutting edge are of first importance. Besides these, however, a certain miscellany of factors involved in microtomy may influence the results and therefore deserve a brief consideration. Examples of such factors are the hardness of the plastic, the way it is trimmed and the rate of cutting.

In our experience with the microtome herein described and with glass knives, the degree of hardness (or brittleness) of the methacrylate determines certain characteristics of the section and sectioning. If the block is excessively hard the sections usually show closely spaced striae (see alternate thick and thin bands in cell nuclei, fig. 6) resulting from a high-frequency vibration ("chatter") at the cutting surface. This "chatter" seems to occur in the plastic block rather than in any component of the microtome because embeddings of softer consistency give little or no trouble in this regard, and in blocks which otherwise cut smoothly, the striae are evident only in the denser components of the tissue such as yolk granules, nuclei or osmium-fixed lipid inclusions. The

excessively hard block is troublesome in a further respect for it considerably shortens the useful life of the glass cutting edge. The unusually soft block has not been so much of a problem although it has been noted that serial sections are more difficult to cut from such embeddings. This appears to result from the ease with which such blocks are distorted by the knife.

In this laboratory consistently good or satisfactory embeddings of soft animal and plant tissues and protozoa have been obtained with pure n-butyl methacrylate catalyzed to polymerization by a compound known as Luperco CDB (produced by Lucidal Division, Noradel-Agene Corp., 1740 Military Road, Buffalo 5, N. Y.). This catalyzer, which is a 1:1 mixture of 2,4-dichlorobenzoyl peroxide and a plasticizer, dibutyl phthalate, is used in concentrations of 2 gm (wet wt.) per 100 cm^3 of monomer. Care has been taken to avoid uncontrolled water contamination of either monomer or catalyst by allowing containers and contents after refrigeration to reach room temperature before opening to room air. The amount of catalyzer may be varied but the hardness of polymer diminishes slightly as more of the plasticizer is included in it. As reported by other workers (Newman, Borysko and Swerdlow, '49), we have found that small percentages (5–10%) of methyl methacrylate mixed with the n-butyl increase the hardness of the block and are useful when an unusually dense or hard material is to be sectioned.

The schedule used in embedding, following dehydration of the tissue, has been as follows:

From absolute alcohol the tissue is transferred to absolute alcohol and n-butyl methacrylate mixed in equal proportions. After one hour there it is taken through three changes of pure n-butyl methacrylate, spaced over two and one-half hours. Thence it is placed in n-butyl methacrylate + catalyst at room temperature for one hour, and finally into fresh n-butyl methacrylate + catalyst (which has been at room temperature) in 00 gelatin capsules. The capsule is filled as close to the top as possible to reduce the relative volume of

enclosed air. Polymerization is carried out at 45°C. and is essentially completed in 24 hours. The block seems to harden somewhat during the next 24 hours, so sections are not generally cut until 48 hours after the reaction has started.

As the first step in sectioning, the gelatin of the capsule is removed by soaking it in H_2O. Thereafter the plastic block is placed in the chuck of the microtome so that about one-quarter inch of it projects beyond the face of the chuck. The convex surfaces of the block are then trimmed with a sharp and clean razor to give the tapered end 4 faces. The taper is made abrupt so that the cutting surface, which is kept small (1–2 mm square), is well braced against distortion. The top and bottom margins of the sectioning surface are kept parallel to give a straight ribbon of sections. With the block in place the front face is trimmed back to the tissue by cutting off several thick sections with the glass knife. For this the knife is advanced to the tissue by turning the bottom screw, at J in figure 1 or 3.

When the trimming has been completed, thin sectioning is started and the block is advanced toward the knife either thermally or mechanically depending on the microtome used. Any striae that appear in the sections may be discerned under the dissecting microscope. The coarser of these running crosswise of the section represent compression waves and foldings which will usually come out if the section is allowed to float for a few minutes on the surface of the 10% acetone used in the section-collecting bath (fig. 1, O). The development of finer striae can sometimes be prevented by moving the face of the block more slowly past the cutting edge.

If sections fail to cut as desired, the reason is sought in one or more of the following sources:

1. The cutting edge may be insufficiently sharp. Evidence of this may appear in the form of knife marks or scratches which run lengthwise through the section.
2. Either the knife or the plastic block may be loose in its holder.

3. The face of the block may be rubbing on the back facet of the knife below the cutting edge. This means that the angle between the knife back and the vertical plane of the block face at the point of sectioning is not the 3–5° that is best.
4. The pivots providing for the support and motion of the horizontal bar may not be adequately tight.

After the sections have rested on the surface of the bath (fig. 1, O) for one or two minutes longer, they are picked up on collodion-coated copper grids (150 openings to the inch). This is done by a spooning motion in which the grid, while held with fine forceps, is placed under the floating ribbon and then lifted out of the bath. The grid and sections are then drained on filter paper and when dry are ready for examination.

Section thickness. As they leave the cutting edge of the knife and float out on the 10% acetone in water, the sections, when properly lighted, display first order interference colors that serve as an index of their thickness. Thus sections are referred to as green, blue, purple, gold and silver and are progressively thinner in that order. Sections of any desired color may be obtained while the horizontal rod is thermally expanded by simply controlling the rate of cutting.

The range of thickness from purple to silver has been found most useful in electron microscopy. Determinations of actual thickness provided by metal shadowing of the lateral margins of the sections show the silver ones to be in the range of 20–50 mμ, the gold 50–80 mμ, and the purple 80–130 mμ. The color analysis of thickness is quite subjective but is sufficient for the practical purpose of deciding whether satisfactory sections are being obtained. With the derived or "improved" microtome (figs. 3 and 4) the advancing mechanism may be set so that consecutive sections 25 mμ thick or multiples of this up to 400 mμ come off the knife.

Results. Since the worth of a microtome is largely determined by the quality of sections it cuts, it seems important to provide some evidence of this quality. Several reports

recently published or in press from this laboratory contain micrographs of tissue sections cut by one or the other of these instruments (Palade, '52b; Porter, '53b; Fawcett and Porter, '53; Robinow, '53). For the most part these have been taken from isolated sections the like of which may be obtained with several microtomes designed for thin sectioning. A more exacting test of the worth of a microtome is whether it can cut a series of several sections of uniform thickness and free of marked distortion.

Put to such a test the instrument described above (fig. 1) has provided material for the micrographs shown in figures 5 and 6. If care is given to trimming the block, to operation of the microtome and to orientation of the ribbon on the specimen holder, preparations of serial sections can be made routinely.

As is obvious to anyone who has attempted the preparation of serial sections for electron microscopy, there are several difficulties apart from the section cutting. Chief among these are (a) the relatively small area of the specimen (ca 1 mm^2) that may be examined in some electron microscopes and (b) the fact that only a small part of the specimen grid itself is open and so available to study.

The first of these difficulties may be met by keeping the length of the sections very small so that any series to be examined will not occupy more than 1 mm on the grid. This is accomplished by reducing the dimensions of the cutting face of the block so that 5 to 10 sections are contained in that length of ribbon.

The second difficulty, i.e., the interference of the solid parts of the grid, is not so readily overcome. One might reasonably make the section length a small multiple of the spacing of the openings, so that the same part of the sectioned tissue would come to lie over open areas. It would of course be necessary in achieving this to orient the ribbon of sections parallel to the solid strands of the grid. A better solution to the problem, however, is to adopt a form of specimen support, at least as far as the opening is concerned, provided

with the Philips, EM 100. It has a slit, 0.2 mm wide and 1.0 mm long. Sections 50 to 100 mμ thick will remain intact over such an opening when they are exposed to the electron beam, provided they are supported on a thin collodion film. Slits of this same sort can be cut in copper foil discs sized to fit the holder of the RCA microscope. With such specimen holders the ribbon of sections must still be oriented parallel to the long axis of the opening but this is quite simply accomplished under a dissecting microscope. In preliminary trials as many as 6 successive sections have been studied. A series of 4 is shown in figures 6A to 6D.

The micrograph in figure 5A shows a section through a binucleate mucous cell of the rat parotid. The plane of the section is oblique but more nearly transverse to the cell than vertical. Secretory capillaries (sc) are evident at the left and bottom margins of the cell with remnants of mucin granules clustered about them. The nuclei (n), mitochondria (m) and the relatively homogeneous matrix of the cytoplasm are readily identified. Besides these there are present numerous "elongated elements" and many "granules" (figs. 5A and 5B, er). These vary from 50 mμ to 150 mμ in diameter and much more in length. When they are examined carefully they are found to have a relatively "transparent" center bounded by a single dense line apparently representing a membrane. These elements of the cytoplasm are easily recognized as the equivalent of an endoplasmic reticulum indicated previously in cultured cells (Porter and Thompson, '48) and identified with the basophilic component of the cytoplasm (Palade and Porter, '52; Porter, '53a).

In thin cultured cells, where the system is spread out more nearly in one plane, it can be seen to consist of a reticulation of strands which are frequently vesiculated. However, its form varies greatly and sometimes includes sinuses which in thin cells are correspondingly flattened. In any further study of this endoplasmic reticulum it is important to determine its three-dimensional form in cells fixed *in situ* in

their respective tissues. Obviously for this and similar problems serial sections of the cells are essential.

From micrographs of such sections then (figs. 6A–6D) it is observed that the system referred to as the endoplasmic reticulum is a complex of interconnected strands and sinusoids, the latter tending to be flattened in one dimension but otherwise very polymorphic. At certain sites in this parotid cell (fig. 5A) these flattened portions of the system are organized in parallel arrays but in other regions of the cytoplasm the system is represented by finer canalicular or vesicular elements (figs. 5A and 5B). If the serial micrographs are examined closely instances may be found where discrete elements appearing as cross sections of canaliculi come together progressively into single elements representing either longitudinal sections through canaliculi or, more likely, marginal sections through sinusoids (figs. 6A–D, er_1). Also in the same series, sequences are apparent defining arborizations of unit structures (at er_2). In still other regions of the cytoplasm (at 3 and 4) the only elements evident appear as tiny vesicles or cross sections of canaliculi. Since certain of these can be traced from section to section (at 3) they are evidently segments of canaliculi.

DISCUSSION

This report has described the construction and operation of two microtomes designed especially for ultrathin sectioning. It has also presented evidence of their capabilities. While these microtomes perform considerably better than three others that have been tried there is no reason to suppose that they cannot be improved upon. However, the experience of the laboratory would suggest that they are adequate for current needs.

Not as much can be claimed for the available knives, which constitute at present the chief limitation to the cutting of thin sections. The cutting edges of glass knives occasionally prove to be excellent but just as frequently they are worthless because of defects which produce scratches in the sections.

This variation in quality is doubtless related to the physical and chemical properties of the glass as well as to the way the knives are cut, but unfortunately precise information on such correlations is not available. We have not examined the properties of steel knives which have been used for thin sectioning by Hillier ('51b) and by Sjöstrand ('53) among others.

There is considerable value in having a microtome which can cut sections thinner than the 100 or 200 mμ previously described as satisfactory. For example, sections 25 to 50 mμ are indispensable for microscopy of the denser components of the cell such as nuclei, chromosomes, yolk granules and intracellular fibers. Certain of these have extremely fine micellar or lamellar structures which cannot be sufficiently well resolved in thicker sections. On the other hand, when one's interest is focused on the general architecture of the cell rather than on structures nearer in size to the limits of electron microscope's resolution, a thicker section, 50 to 100 mμ, is more useful. This is especially true in the serial section example presented above (figs. 6A–6D). What the micrographs of these thicker sections lose in resolution they gain in "depth" of morphology depicted. There is, however, a distinct limit to this advantage for in sections thicker than 150 mμ the overlapping of cell components and the diffuseness of margins as a consequence of greater electron scatter result in an abrupt decrease in the worth of the pictures. The micrographs of the very thin and to a lesser extent the thicker sections lack the contrast shown by the 50 to 100 mμ sections.

The problem of contrast is a companion of the several others involved in thin section microscopy. It has been dealt with in several ways. One procedure has been to dissolve out the embedding matrix and thereafter shadow. This obviously makes for greater density differences in the specimen, but at the same time, as Hillier and Gettner ('50) have indicated, it leads to additional artefact development. Obviously when the matrix is removed some of the finer, unattached

components of the tissue section go with it. Furthermore, as the liquid-air phase boundaries pass through the section in drying, many of the elements of the cell are moved to the larger fixed components by the forces of surface tension. It follows, therefore, that where reasonably faithful preservation of native fine structure is required the methacrylate matrix should be left in place. Fortunately, the mass density of the methacrylate may be sufficiently reduced by the more convenient and less destructive procedure of simply exposing the section to the electron beam. Apparently some component or components of the plastic are sublimed off while the density and distribution of the tissue elements are essentially unaffected. Thus the contrast in the image is greatly enhanced and is entirely adequate if the objective aperture in the microscope is sufficiently small.[1]

With adequate procedures available for the fixation (Palade, '52a), dehydration, embedding and sectioning of biological material for electron microscopy it is excusable to show

[1] Since electron microscopy of thin sections requires the use of apertures a few notes from our practical experience in this regard seem worth including.

We have found with the RCA EMU that a 25 μ objective aperture in a platinum diaphragm provides adequate contrast with sections of average thinness (50 mμ–100 mμ). This opening is smaller than the one generally used and may be troublesome to center when first installed. If, however, the orientation of the diaphragm with respect to the pole piece is recorded and the diaphragm is not bent or otherwise mutilated during cleaning, it can often be replaced in the column with only one or two minor adjustments of the centering screws. A condenser aperture of 0.30 mm and an intermediate 1.4 mm opening as part of the old intermediate viewing screen (supplied with EMU, 2B) are also part of the aperture setup in this microscope, but compared with the smaller objective aperture, these latter are of little consequence.

Contrast-wise the above setup is as effective as the newer wide-bore objective pole piece and multiple apertures (Hillier, '51c) that we have tried (with intermediate lens) in another microscope of the same type. This latter assembly gives, however, a much greater magnification range and is therefore valuable for tissue-section microscopy where low power image study is often essential.

The contrast in the micrographs in figures 5 and 6 was obtained with the use of a 40 μ aperture in a Philips EM 100 operating at 60 KV. The variable voltages of this microscope are also useful for obtaining uniform contrast in spite of variations in section thickness. Thus the lower voltage (40 KV) is employed for extraordinarily thin sections and the higher voltage (80 and 100 KV) for the exceptionally thick ones.

some enthusiasm over the possibilities open to students of cell and tissue structure. Many of the relatively static forms and relationships of cells which heretofore have been hidden just at or beyond the limits of light resolution are now being studied. Even the more protean intracellular structures of which several have defied optical analysis can reasonably be explored. That such studies are both possible and productive is amply demonstrated by current publications.

Far from being futile then, as once expressed by Hillier ('51a), the analysis of cell morphology by these techniques is a fruitful reality. It is true that to describe precisely the fine structure of a single parotid cell would require the cutting and study of approximately 200 serial sections. However, there would be little purpose in giving that much attention to one cell, for it might conceivably be peripheral in the normal range of morphology shown by the secretory cells of this gland. It is far better and easier to reconstruct a typical cell from the images of many presented at various angles and aspects within a few sections of the tissue. Only in the analysis of the form and structural relationships of small organelles, as in the example presented above, are serial sections essential. For this, however, the number required would not be enormous. It should not be decided either that because a structure is fixed or lifeless when studied, one cannot from an examination of a reasonable amount of material reconstruct in submicroscopic dimensions the story of it development or change with functioning. These are processes which can be related to some phase or cycle of change known through light microscopy of living material. All that is necessary for their study is that the techniques of specimen preparation and microscopy be sufficiently good and sufficiently simple for observations to be made with reasonable speed and accuracy.

In this report special attention has been given to a submicroscopic component of the cytoplasm referred to as the endoplasmic reticulum. Besides illustrating the worth of serial sections it exemplifies a type of intracellular structure

of intermediate fineness which heretofore has been just beyond the limits of direct observation.

This newly defined system of the cytoplasm is described above as a complex reticulum of connected strands and sinusoids. Evidence has been presented elsewhere (Porter, '53a) that the system coincides in distribution with the basophilic component of the cytoplasm and it is assumed that it represents or is closely associated with this component of cell structure. It seems moreover to be the main component of the so-called microsomal fraction obtained from cells by differential centrifugation. Obviously it provides the cytoplasm with a membrane-bound system of relatively enormous surface area presumably important to enzyme-controlled reactions. There is reason to believe also that the degree of reticulation and surface area change greatly with shifts in the physiological activity of the cell. Certainly in changes preceding cytolysis there is a marked vesiculation of the system's strands and sinusoids. For a more complete discussion of this system in cells *in situ* reference should be made to Palade ('54).

SUMMARY

Two new microtomes capable of cutting serial sections as thin as 25–50 mμ are described. All moving parts in these instruments are supported in unlubricated pivots and the specimen is taken past the cutting edge only on the cutting stroke. These two features more than any others seem responsible for the successful performance of these microtomes. In one instrument, the prototype, the block is advanced to the knife by thermal expansion. In the other, the advancement is controlled mechanically. Procedures involved in specimen preparation, especially those that influence the quality of the sections, are also described. Evidence of the capabilities of the microtome is presented in micrographs of 4 serial sections of rat parotid showing, in a portion of a single cell, the three-dimensional distribution of the endoplasmic reticulum.

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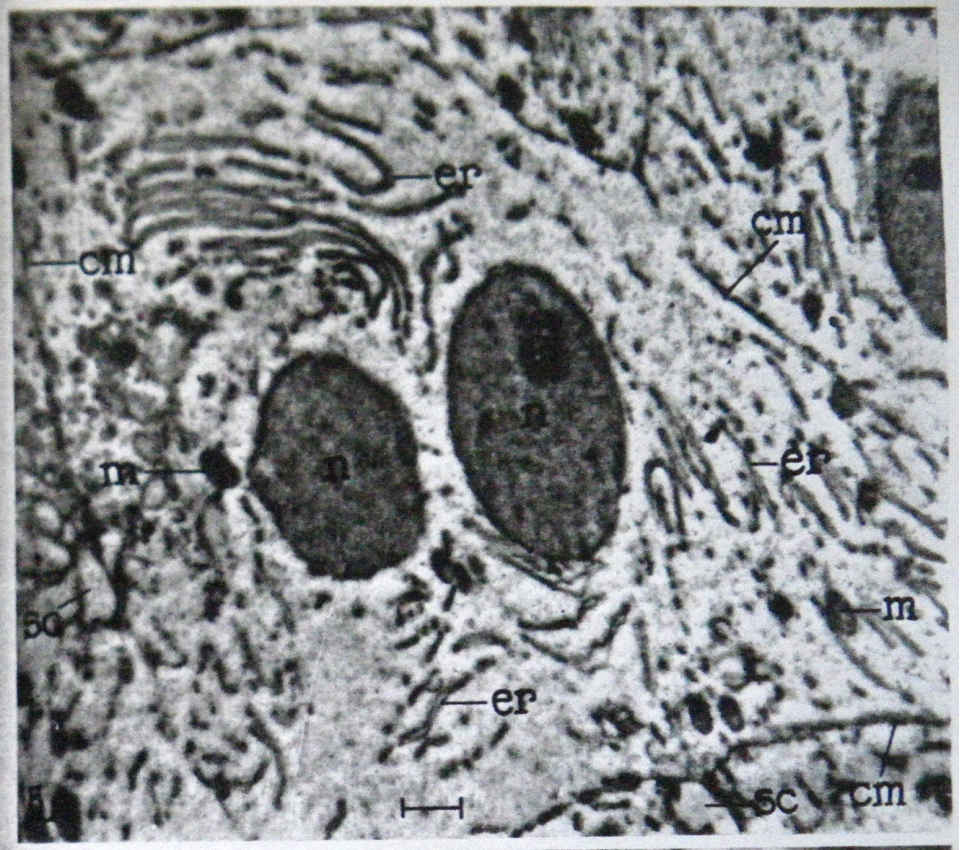
PLATE 1

EXPLANATION OF FIGURES

5A Electron micrograph from a thin section of adult rat parotid. The tissue was fixed in buffered 1% OsO_4 for 30 minutes. The greater part of the picture is occupied by a single, binucleate mucous cell. The cell boundaries (cm) and secretory capillaries (sc) are indicated. The two nuclei (n) are dense and include parts of nucleoli which are even more dense. A few mitochondria (m) are evident but the dominant elements of the cytoplasm are slender, membrane-bound structures looking like longitudinal sections through canaliculi or flattened sinusoids (er). Less prominent and smaller granular elements also present are evidently cross sections through canaliculi or vesicles. Most of these are parts of the endoplasmic reticulum (er) described previously in cultured cells. Some information on the distribution and form of this component in the depth dimension may be gleaned from a comparison of the micrographs in figure 6, which show a small part of 4 consecutive sections through this same cell. Micrograph was taken with the Philips EM 100 at 1860 diameters and enlarged here to × 8,000.

5B This is a higher resolution micrograph of a part of the section pictured in figure 5A and presented to show in greater detail the components of the endoplasmic reticulum (er_1). The elongated elements which are the more prominent are defined by dense lines, presumably representative of membranes. The enclosed spaces or lumina appear free of content. These structures doubtless represent longitudinal sections through canaliculi or transverse sections through thin or flattened sinusoids. The less prominent, oblong and circular images (er_2) have the same morphology but appear to represent cross sections through canaliculi or small vesicles of the same reticular system. A mitochondrion is evident at (m) and a nucleus at (n). Philips micrograph, taken at 3400 and enlarged here to × 20,000.



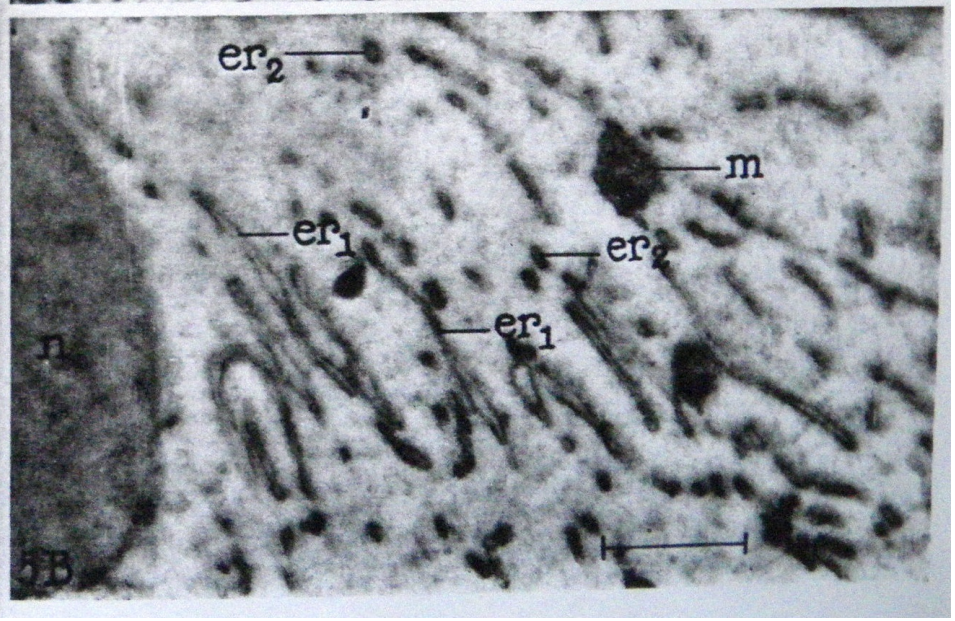
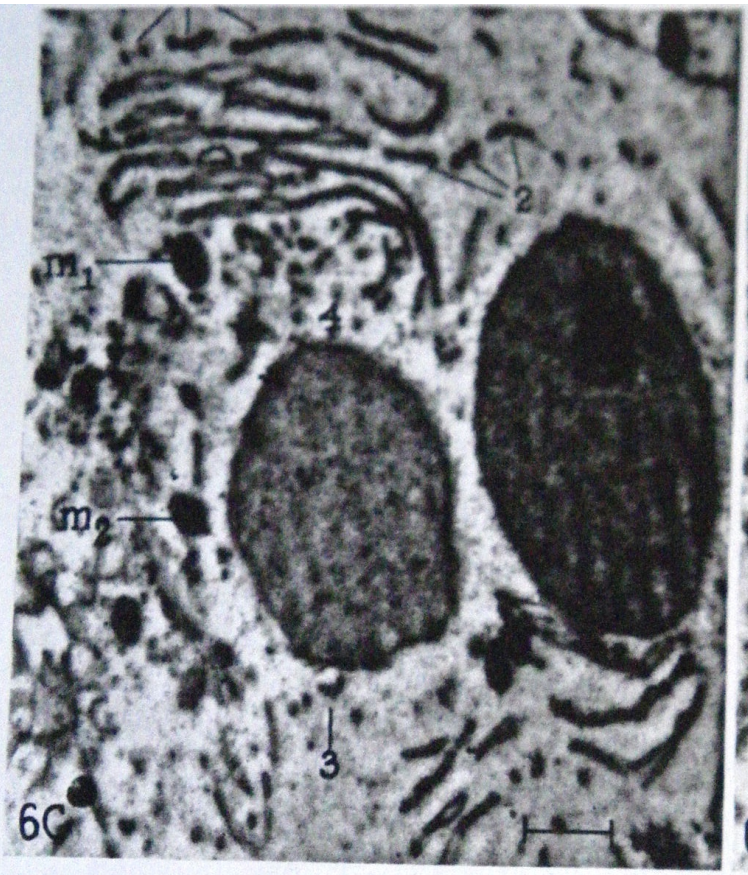
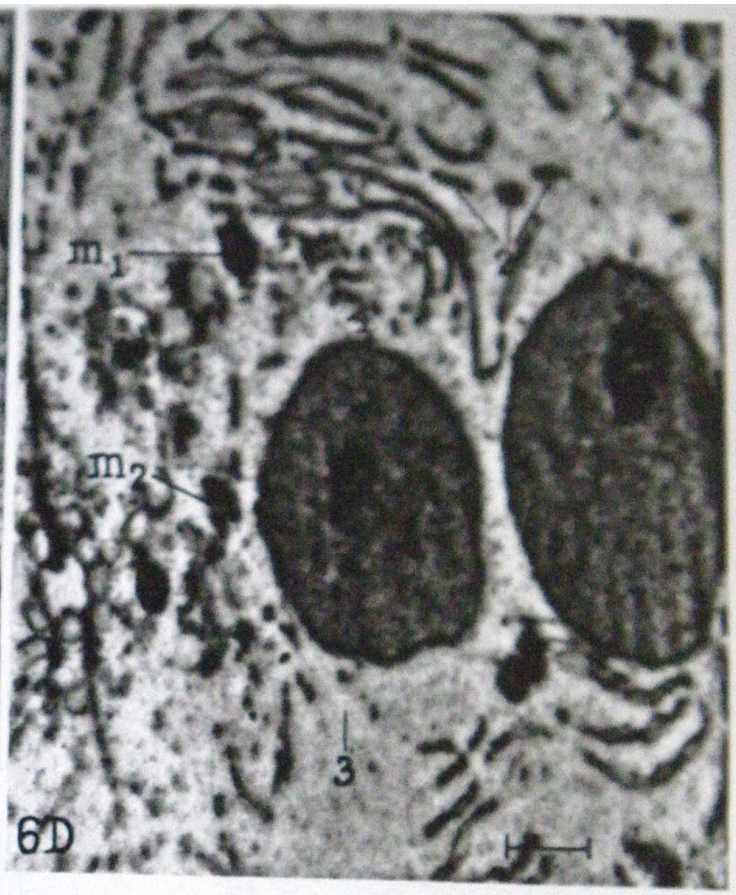


PLATE 2

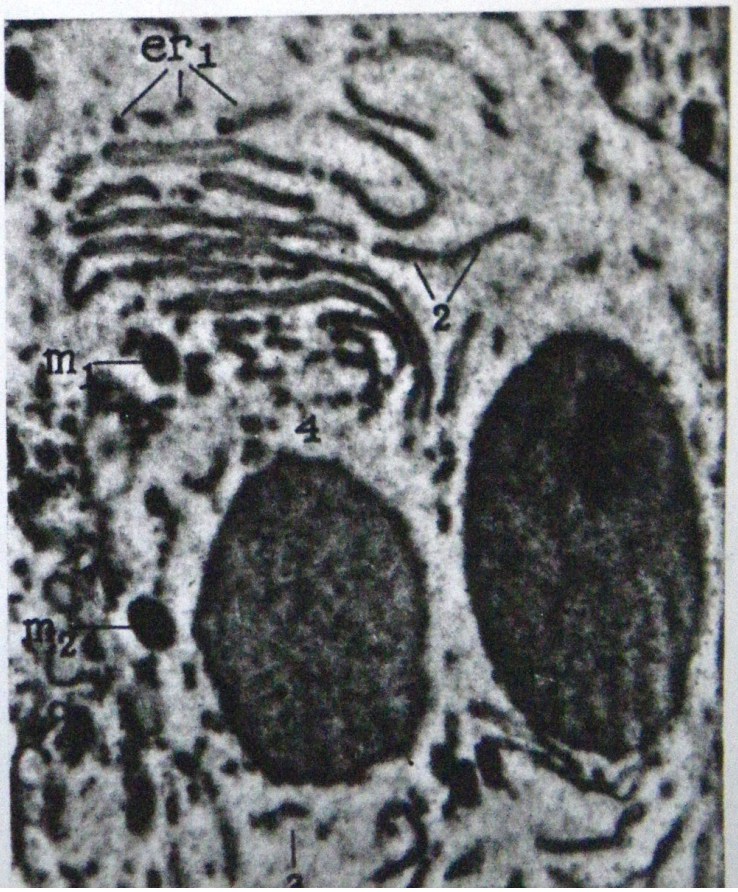
EXPLANATION OF FIGURES

6A to 6D Corresponding portions of 4 consecutive sections. The micrographs were taken at a magnification of 1860 diameters with the Philips EM 100 and enlarged here 5.9 times. A total thickness of about 300 mμ is represented by the 4 sections. It is possible to trace structures such as mitochondria (m) and elements of the endoplasmic reticulum (er) through successive sections and observe shifts in their size, position and orientation at different levels. Starting with 6A it can be seen that mitochondrion, m_1, is represented only by a tangential section through its end or periphery. In 6B, C and D, it gains in prominence as the plane of the section advances further into the organelle. Mitochondrion, m_2, on the other hand, is gradually lost in the same sequence. Portions of the endoplasmic reticulum likewise change form at different levels. These changes can be followed best at $er_{1,2,3,4}$. At er_1 a number of elements that are independent in 6A appear in elongated form in 6B and 6C and seem to have fused at the level of 6D. This indicates that three or more strands at level 6A run together into a common strand or sinus at 6D. Er_2 points to a single strand or body in 6A, which divides into at least 4 separate elements by the level of 6D. In the region indicated by (3) a number of circular elements may be traced through all 4 sections and doubtless represent transverse sections through canaliculi. A confusion of similar images above (4), of which some begin and end in the depth of the 4 sections, seems to represent sections through small independent vesicles. Mag. × 11,000.


2
m1
4
m2
3
6C


m1
m2
3
6D


en1
2
m1
4
m2
3


er1
2
m1
4
m2
3